THE STABILITY OF ANEURINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS

By H. Partington and C. E. Waterhouse

From the Research Department, Bob Martin, Ltd., Southport.

Received July 13, 1953

It is well known that aneurine hydrochloride, in addition to being heat-labile, is also destroyed when maintained in media having either neutral or alkaline *p*H, with the formation of decomposition products having no vitamin B_1 activity. It has been shown by Farrer[1] that there is, for any given buffer solution, a linear relationship between the reaction velocity of the destruction of aneurine and the hydrogen ion concentration in the *p*H range 3 to 8. In all cases, the velocity of the reaction was found to increase as the *p*H rose. In a later paper Farrer[2] has shown that the concentration of buffer salts affected the rate of destruction of aneurine below *p*H 6, and that the rate of destruction is also dependent on the initial concentration of aneurine. This work was mainly concerned with thermal destruction of the vitamin in solutions, and the present paper records the results of investigations of the stability of aneurine hydrochloride in pharmaceutical powders containing either semimicro quantities or higher concentrations, and also in tablets, when these are stored at normal temperatures over a period of years. It would be expected that vitamin decomposition in a relatively dry, non-hygroscopic powder would be very much less than in a solution under comparable conditions of acidity, etc. This has, in general, been found to be the case, although significant loss of vitamin occurred even in these relatively dry powders in the least acid media. The observations were carried out on samples taken from routine production and usually involving fairly large batches of material. Large numbers of routine determinations have been carried out and some 110 samples of the various formulations were selected for special study.

Analytical Methods

The analytical method used for the determination of aneurine hydrochloride throughout the series followed that laid down in the British Pharmacopœia, 1948, Appendix XIII. With the exception of certain of the earlier results, which are referred to subsequently, the photoelectric method was used employing the Spekker Fluorimeter, the only deviation from this method being that where necessary, when dealing with buffered materials, the proportion of hydrochloric acid employed to dissolve the sample prior to oxidation was somewhat increased. The increase was carried out only to the extent necessary to give a residual *p*H of approximately 2 prior to the oxidation, and at this stage the solution was therefore comparable to the B.P. solution employed when assaying the pure aneurine hydrochloride. The thiochrome method has been the subject of numerous papers and has been criticised, especially for use with larger concentrations

of aneurine, but it has been our experience that this method yields results, at the concentrations dealt with, well within the precision of ± 5 per cent. quoted by Adamson and Handisyde[3], who preferred a gravimetric method for the estimation of higher concentrations to avoid large dilutions.

In examining pharmaceutical preparations such as capsules, syrups and elixirs, Elvidge[4] found it necessary to use an adsorption technique on a synthetic zeolite, but he was able to use the direct acid extraction method for tablets. As indicated above, we have also used the direct extraction method for all the powders and tablets under study.

Where a figure for the *p*H of the samples is quoted, this refers to the *p*H, determined electrometrically, of a 2 per cent. w/v solution, or suspension, of the sample in distilled water, the determination being carried out after a stable value had been reached. This is, of course, an arbitrary figure, and does not necessarily represent the conditions obtaining in the relatively dry powder, which is the environment in which any decomposition during storage has actually occurred. The *p*H figures quoted are useful, however, in classifying the various samples examined.

Experimental Results

All the samples tested had been stored under the following conditions, and for the period of time indicated in the tables below. The powders or tablets were in glass containers, corked, but not hermetically sealed and not full: there was, therefore, a limited amount of atmospheric oxygen in contact with the sample. They were stored almost the whole time in the dark, at a temperature which varied between 60° and 70° F., and in relatively dry external atmospheres with relative humidity not exceeding 65 per cent.

Group 1. Formula: aneurine hydrochloride 15 I.U. per 5 grains, in a base of lactose, in presence of calcium phosphate B.P., ferric phosphate, manganese lactate, copper sulphate.

The initial analyses were carried out by the visual method for the fluorimetric assay of aneurine hydrochloride, described in the Appendix XIII of the B.P. 1948. It will be seen by comparison with later results for the initial analyses, that although the standard deviation is much greater than in groups where the photoelectric method was employed, the analysis is none the less remarkably near to the correct figure, having in mind the limitations of the older visual method. There is, of course, a somewhat large standard deviation in the results for the final analysis but this includes not only assay variations but also the effect of a slightly variable rate of vitamin decomposition among the individual samples in the group under study.

Group 2. Formula: aneurine hydrochloride 15 I.U. per 5 grains, in a lactose base, in presence of ingredients as for Group 1, with the addition of calcium gluconate B.P.

In this case also half the initial determinations of aneurine content were carried out by the visual method and half by the photoelectric method, the latter method being commenced in October, 1948, and continued throughout all the remaining determinations. The smaller standard deviation in

the results for the final analysis is indicative of a more uniform rate of vitamin loss in the samples of this group.

Group 3. Formula: aneurine hydrochloride 31 I.U. per 5 grains, in a lactose base, in presence of nicotinic acid, calcium phosphate, calcium gluconate, ferric phosphate, copper sulphate, cobalt lactate.

This formulation, having a *p*H value of 4·5 and being fairly highly buffered, gives, as would be expected, greater vitamin stability. It is interesting to note, however, that the average decrease in vitamin content is more or less the same for materials stored for either 1, 2, or 3 years. This would seem to indicate an initial small loss of vitamin activity within a fairly short period of manufacture, followed by little further loss when stored for longer periods.

TABLE I

POWDERS CONTAINING LOW CONCENTRATION OF ANEURINE HYDROCHLORIDE

Group	Date of preparation of material	Initial aneurine content by analysis	Storage period	Aneurine content after storage	Average decrease in aneurine content per cent.	*p*H of 2 per cent. suspension
1	From September, 1947, to August, 1948.	14·6 I.U. Standard Deviation ± 2·57	4½ years	9·8 I.U. Standard Deviation ± 2·04	33	6·5
2	From September, 1948, to July, 1949.	14·5 I.U. Standard Deviation ± 2·05	3½ years	11·6 I.U. Standard Deviation ± 1·04	19·7	5·5
3 (*a*)	From August, 1949, to August, 1950.	30·9 I.U. Standard Deviation ± 0·224	3 years	28·4 I.U. Standard Deviation ± 1·4	8·2	4·5
3 (*b*)	From September, 1950, to August, 1951.	31·8 I.U. Standard Deviation ± 1·22	2 years	28·4 I.U. Standard Deviation ± 0·81	10·8	4·5
3 (*c*)	From September, 1951, to August, 1952.	31·7 I.U. Standard Deviation ± 1·47	1 year	29·3 I.U. Standard Deviation ± 1·44	7·5	4·5
4 (*a*)	From January, 1948, to March, 1949.	34·3 I.U. Standard Deviation ± 2·56	2 years	31·1 I.U. Standard Deviation ± 1·83	9	6·0
4 (*b*)	From January, 1948, to March, 1949.	34·3 I.U. Standard Deviation ± 2·56	4 years	29·5 I.U. Standard Deviation ± 2·07	14	6·0

Group 4. Formula: aneurine hydrochloride 35 I.U. per 5 grains, in a lactose base, in presence of calcium gluconate, calcium phosphate, ferric phosphate.

In this group an observation of the aneurine content of each sample under study was made at the end of 2 years, in addition to the recent analysis after 4 years. The average decrease at 9 per cent. in the first 2-year period, compared with 5 per cent. in the second 2-year period, is of considerable interest and suggests that a detailed graph of vitamin loss under these conditions would be exponential in form. Another point of interest here is that in the absence of traces of any heavy metals, such as copper, the average rate of vitamin loss, even at *p*H 6·0, under these conditions is less than the average loss at *p*H 5·5 over a similar period in the presence of these metals.

Group 5. Formula: A simple mixture of aneurine hydrochloride with Lactose.

From these determinations it is interesting to note that in simple

aneurine mixtures at higher concentrations, it is possible to obtain analytical results within quite narrow limits, as evidenced by the low standard deviation. It is interesting also to note the relatively small average decrease in aneurine content at this *p*H, and a number of the figures are reproduced in Table II to show that loss of vitamin content does not appreciably increase with increased age of sample at this concentration and under these conditions.

TABLE II

POWDERS CONTAINING HIGHER CONCENTRATION OF ANEURINE HYDROCHLORIDE

Group	Date of preparation of material	Initial aneurine content per cent.	Aneurine content, at January, 1953 per cent.
5 (*a*)	Batch No. 8, January, 1950	0·81	0·71
5 (*b*)	Batch No. 14, June, 1950.	0·81	0·78
5 (*c*)	Batch No. 18, September, 1950 ..	0·80	0·76
5 (*d*)	Batch No. 22, December, 1950 ..	0·81	0·78
5 (*e*)	Batch No. 27, June, 1951	0·79	0·76
5 (*f*)	Batch No. 31, August, 1951	0·79	0·78
5 (*g*)	Batch No. 35, January, 1952.. ..	0·82	0·76
5 (*h*)	Batch No. 39, July, 1952	0·78	0·79
5 (*j*)	Batch No. 42, October, 1952 ..	0·79	0·73

Average initial aneurine content: 0·80 per cent.; Standard Deviation 0·013.
Average final aneurine content: 0·76 per cent.; Standard Deviation 0·027.
Average decrease in aneurine content: 5 per cent.
*p*H of 2 per cent. solution: 3·8.

Group 6. Formula: aneurine hydrochloride 15 I.U. per 3 grains, in a lactose base, in presence of calcium and ferric phosphates, heavy magnesium carbonate, starch, acacia, talc.

In the majority of samples, re-analysis after 6 years showed no vitamin detectable at the limit of sensitivity of the fluorimetric assay. One sample gave approximately 2·5 I.U. per tablet remaining, and another approximately 1 I.U. per tablet. The complete decomposition of the vitamin in a medium in which the moisture content does not exceed 2 per cent. shows that the observation of Farrer[1] and others on the decomposition of aneurine in alkaline solutions applies also to alkaline powders and tablets. The loss of vitamin potency might not be expected in relatively dry media where the total moisture content does not exceed 2 per cent., and where the alkali present is normally regarded as "water insoluble." It is evident that aneurine is very sensitive to traces of alkali.

Group 7. Formula: aneurine hydrochloride 15 I.U. per 3 grains, in a lactose base, in presence of calcium and ferric phosphates, starch, acacia, talc.

This formula is similar to that of Group 6, but without the mild alkalinity contributed by the magnesium carbonate.

Group 8. Formula: aneurine hydrochloride 15 I.U. per 3 grains, in a lactose base, in presence of calcium gluconate, ferric phosphate, starch, acacia, talc.

There was small difference in formulation between the two groups quoted. Batches manufactured from July, 1948, to December, 1948, contained 10 per cent. of calcium gluconate, and those from January, 1949, to July, 1949, contained 20 per cent. of calcium gluconate. Although the *p*H of the suspension in both groups was 5·5, the presence of double the

quantity of calcium gluconate in the latter group may well have been responsible for the much smaller decrease in vitamin content compared with the first series.

Group 9. Formula: aneurine hydrochloride 34 I.U. per 3 grains, in a lactose base, in presence of calcium gluconate, ferric phosphate, nicotinic acid, starch, acacia, talc, and small quantities of cobalt lactate and copper sulphate.

Due to the presence of 0·5 per cent. of nicotinic acid in this formula, the resulting *p*H of the suspension has moved down to 5·0. The average

TABLE III

TABLETS CONTAINING LOW CONCENTRATION OF ANEURINE HYDROCHLORIDE

Group	Date of preparation of material	Initial aneurine content by analysis	Storage period	Aneurine content after storage	Average decrease in aneurine content per cent.	*p*H of 2 per cent. suspension
6	From October, 1946, to June, 1947.	15 I.U.	7 years	2·5 I.U. 1·0 I.U.	88	8·5
7	From October, 1947, to May, 1948.	15 I.U.	5 years	12·9 I.U. Standard Deviation ± 0·77	14	7·0
8 (*a*)	From July, 1948, to December, 1948.	16·3 I.U. Standard Deviation ± 1·31	4 years	13·25 I.U. Standard Deviation ± 0·375	18·7	5·5
8 (*b*)	From January, 1949, to July, 1949.	15·5 I.U. Standard Deviation ± 0·57	3½ years	14·5 I.U. Standard Deviation ± 0·515	7·2	5·5
9	From July, 1949, to October, 1950.	33·1 I.U. Standard Deviation ± 2·4	2½ years	29·9 I.U. Standard Deviation ± 1·05	9·6	5·0

decrease in aneurine content is, however, similar to that in the second series of tablets considered under Group 8, where the *p*H was 5·5. The vitamin loss at these *p*H values is like those recorded in Group 3 for powders—approximately 10 per cent. over a fairly wide range of storage periods.

It is evident that to produce a preparation with virtually no loss over long storage periods, a *p*H of tablet in the region of 4, comparable with the powders quoted in Group 5, must be aimed at.

THE INFLUENCE OF COPPER ON THE RATE OF ANEURINE DESTRUCTION

The effect of copper in buffer solutions at various *p*H when heated at 100° C. was studied by Farrer[5]. He used copper sulphate giving Cu concentrations of 2 and 20 p.p.m. in the solutions under test. In phosphate and phosphate/phthalate solutions copper accelerated the rate of destruction of aneurine at all *p*H values studied, greater acceleration being obtained with the greater concentration of copper. The effect was modified where copper was in the presence of radicals with which it could form complex ions, such as tartrate or citrate.

The tablets to the formulation quoted in Group 9 contained copper sulphate giving a Cu concentration of 87 p.p.m. It will be seen that even in the presence of this relatively high concentration of copper there was an average vitamin loss of only 9·6 per cent. after 2½ years

storage. The loss of 9 per cent. of the vitamin content in presence of so much copper, and at *p*H 5·0, compares with a similar loss of 9 per cent. recorded after 2 years in the formula under Group 4. This formula contains no copper and has a similar base of gluconate/phosphate, but no nicotinic acid.

The powders to the formulation recorded in Group 3 contained an even higher proportion of copper sulphate, giving a Cu concentration of 202 p.p.m. This formulation, having a *p*H value of 4·5, shows a vitamin loss of only 8 to 10 per cent. after as long as 3 years. This concentration of copper is 10 times as great as the higher of the two concentrations used by Farrer. It is evident, therefore, that the effect of copper is greatly diminished in dry powders containing phosphate/gluconate buffers, when stored at room temperatures over long periods.

Summary

1. Compound powders and uncoated tablets containing various concentrations of aneurine hydrochloride have been re-assayed after storage for a number of years.

2. Data are given to show the range of vitamin contents for the various formulæ as originally manufactured and after storage. The results indicate that in these relatively "dry" media aneurine is quite stable for long periods at *p*H 4. In formulæ of *p*H 4·0 to 5·0 there is a loss of the order of 10 per cent., most of which occurs relatively soon after manufacture. In media of *p*H greater than 5 there is correspondingly greater loss, until at *p*H 6·5 there may be a loss, after a period of years, of up to 33 per cent. At higher *p*H values, practically the whole of the vitamin content eventually disappears. These losses follow the pattern of the behaviour of aneurine in solution and during thermal decomposition, but in media of low moisture content decomposition tends to be less rapid.

3. The effect of the presence of proportions of heavy metals, such as copper and cobalt, has been examined in the light of their known effect on solutions containing the vitamin. It has been found that with relatively high concentrations of copper there appears to be little effect due to these metals on the vitamin loss under the conditions studied. It appears, therefore, that the effect of copper is greatly diminished in dry powders when stored at room temperatures over long periods.

We should like to acknowledge the assistance of Miss M. Hardaker in carrying out certain of the analyses.

References

1. Farrer, *Biochem. J.*, 1945, **39**, 128.
2. Farrer, *ibid.*, 1947, **41**, 167.
3. Adamson and Handisyde, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 370, 423.
4 Elvidge, *ibid.*, 1947, **20**, 257.
5. Farrer, *Biochem. J.*, 1947, **41**, 162.

DISCUSSION

The paper was presented by MR. C. E. WATERHOUSE.

MR. N. L. ALLPORT (London) said that he had analysed tablets containing exsiccated ferrous sulphate and calcium carbonate to which aneurine hydrochloride had been added, and had found that the content of aneurine hydrochloride was much below that claimed. He was surprised to find that the authors were apparently satisfied that so long as the components were dry, the iron present did not interfere. The authors did not specifically mention exsiccated ferrous sulphate but they did refer to ferric phosphate, and he wondered whether calcium carbonate mixed with ferric phosphate would cause a serious deterioration in the aneurine content.

DR. K. BULLOCK (Manchester) quoted results of his own experiments using a spray dried powder of ascorbic acid containing traces of copper. The catalytic activity of the copper was inhibited while the powder remained dry, but in solution the ascorbic acid was oxidised rapidly. He had found that moisture had considerable effect and asked the authors for more details about the moisture content of their powders and how it was determined. In powders which contained starch or hydrated salts the question of whether the moisture was free or bound would arise. He presumed that it was the free water which would take part in the various reactions. In the summary the authors pointed out that the results indicated that in relatively dry media aneurine was quite stable for long periods at *p*H 4. He had given considerable thought as to what should be meant by the *p*H of dry powders. The normal meaning of the term *p*H was, of course, based on dilute solutions, and what was meant by *p*H in powder seemed to be difficult to decide. In order to compare figures for storage, it was necessary to have some concept of how the rate of decomposition varied with time, and he wondered whether the authors had any figures to show whether the decomposition rate was linear, logarithmic or followed some other law.

DR. R. E. STUCKEY (London) said that he also was interested to note that the authors found most of the tablets to be stable even though in almost every case they had an appreciable iron content. He had found that in the presence of ionised ferric iron, aneurine was distinctly unstable. Could the authors state whether ferric ions would be present when their tablets which contained ferric phosphate and various calcium salts were taken up in water? In tablet form ferrous sulphate reacted with calcium carbonate forming calcium sulphate which set hard, like plaster of paris, and the water released would probably catalyse decomposition.

MR. T. D. WHITTET (London) said that he had made injection of aneurine hydrochloride B.P. 1953 but after 6 to 12 months' storage a distinct precipitate had formed. When the samples were assayed they gave full theoretical values and it would be interesting to know whether the authors had had any similar experience.

DR. F. WOKES (King's Langley) referred to the stability of different forms of aneurine, and said there was a good deal of evidence to suggest

that aneurine in its natural complexes was considerably more stable than synthetic aneurine hydrochloride. During the last 10 or 11 years he had been responsible for standardising the aneurine content of preparations containing partly natural aneurine, no doubt in complex, and partly added synthetic aneurine. He had found stabilities in those preparations tested over periods of years to be higher than those recorded in the paper. The moisture contents in some cases were between 1 and 2 per cent., but there were also preparations containing about 20 per cent. moisture which were of comparable stability. The *p*H was between 4·8 and 5 and iron was present.

MR. C. E. WATERHOUSE, in reply, said that trouble had been experienced with powders which contained magnesium carbonate, another insoluble and alkaline material. He felt that in Mr. Allport's case the trouble might be due more to the calcium carbonate than the iron present. In passing, he observed that on examining a commercial powder stated to contain 50 I.U./g. of aneurine, and magnesium carbonate, it was found to contain only 15 I.U./g. The references to the depression of the catalytic activity of copper in low moisture systems were interesting. It was well known that copper in solution caused fairly rapid loss of the vitamin. The moisture content of the materials under discussion was of the order of 0·2 to 0·5 per cent. for powders and 1·5 to 2 per cent. for tablets. The meaning of the term *p*H in reference to dry powders was stated in the paper. On the question of storage, he explained that the data set out had only been accumulated as parts of other investigations and routine tests over the years, and the paper was a record of the results obtained. The iron present was in the form of ferric phosphate and was, as far as one could say, in an insoluble form. It was, therefore, quite different in its effect from ferrous sulphate mixed with calcium salts. He was unable to comment on the point raised by Mr. Whittet. He agreed that natural aneurine might be more stable than synthetic, but he had shown that the synthetic vitamin was very stable over a long period of time provided that the formulation and the ultimate *p*H of the substances present were carefully studied.